AD NUMBER

AD866105

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; Feb 1970. Other requests shall be referred to Technical Release Branch [Army], Fort Detrick, MD 21701.

AUTHORITY

BDRL d/a ltr, 29 Sep 1971

AD

AD 866105

TECHNICAL MANUSCRIPT 574

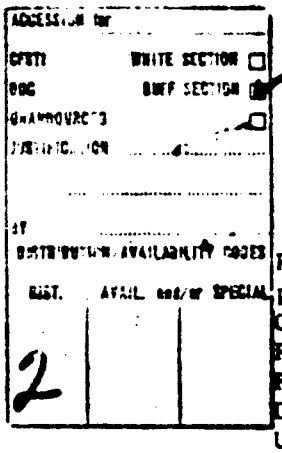
AN UNUSUAL BIPHASIC DEATH RESPONSE IN MICE INFECTED WITH ATTENUATED PASTEURELLA PESTIS

Earl D. Beesley
Michael J. Surgalla

FEBRUARY 1970

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

49564



DDC AVAILABILITY NOTICES

Qualified requesters may obtain copies of this publication from DDC.

Foreign announcement and dissemination of this publication by DDC is not authorized.

Release or announcement to the public is not authorized.

DISPOSITION INSTRUCTIONS

Destroy this publication when it is no longer needed. Do not return it to the originator.

The findings in this publication are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

TECHNICAL MANUSCRIPT 574

AN UNUSUAL BIPHASIC DEATH RESPONSE IN MICE INFECTED WITH ATTENUATED *PASTEURELLA PESTIS*

Earl D. Beesley

Michael J. Surgalla

Medical Investigation Division
MEDICAL SCIENCES LABORATORIES

Project 1B662706A072

February 1970

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ABSTRACT

The classical nonpigmented (P^-) plague vaccine strain EV76, which is unable to absorb hematin and exhibits very low virulence for the white laboratory mouse by the intraperitoneal and subcutaneous routes, has been found to be highly virulent when injected intravenously. The lethal dose response curve is biphasic, suggesting that doses in the intermediate range of 10^4 to 10^6 organisms stimulate an effective host resistance mechanism resulting in survival of most of the mice. This peculiar virulence pattern is shown also by nonpigmented variants of strains Kim-10 and M23.

The negative response phase (reflected by survival of infected animals) is less pronounced in mice infected with Fraction I minus (FI^-) organisms such as M23 (P^-FI^-) and Kim-10 (P^-FI^-), suggesting a relationship to FI. Two suggested possible stimuli of the effective host resistance are FI and endotoxin.

AN UNUSUAL BIPHASIC DEATH RESPONSE IN MICE INFECTED WITH ATTENUATED PASTEURELLA PESTIS*

Pasteurella pestis, the causative agent of bubonic and pneumonic plague, has been studied intensively and a great deal has been learned concerning the relationship between certain phenotypic characteristics and the degree of virulence of various strains. A brief generalization of these relationships is shown in Table 1. This report is concerned with the last three types of organisms listed there, and describes an unusual death pattern in mice infected with any of these organisms.

In 1926 Girard and Robic[1] found that one of their isolates, strain EV76, was attenuated for laboratory animals and man. Since then this strain has been used to immunize millions of people in Madagascar, Africa, Russia, and Southeast Asia. Characterization of EV76 by numerous workers has revealed that its low degree of lethality is associated with the loss of the pigmentation character. The absence of this virulence-related characteristic is recognized by the organism's inability to absorb hematin or Congo red dye from agar media. Typical pigmented and nonpigmented organisms are shown in Figure 1. On the synthetic medium of Jackson and Burrows[2] nonpigmented (P^-) organisms appear straw colored and pigmented (P^+) colonies are dark brown. Similar results are obtained with a simplified preparation (Congo red agar) developed in our laboratory.[3] P^+ organisms appear bright red and P^- colonies are only slightly pink (Fig. 2).

P^- organisms are of very low virulence for animals and man under most circumstances. Burrows has demonstrated, however, that injection of small amounts of iron enhances lethality for the mouse and, to a lesser extent, the guinea pig.[3]

Figure 3 illustrates the death pattern caused by EV76 when injected by the intraperitoneal route. Relatively large numbers of cells fail to cause death. When iron is injected concomitantly a marked lethality is seen even at the lowest dose.

---

* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the senior author to ascertain when and where it may appear in citable form.

TABLE 1. RELATIONSHIP BETWEEN PHENOTYPIC CHARACTERISTICS AND LETHALITY OF TYPICAL STRAINS OF PASTEURELLA PESTIS

Investigator	Strain	VW[a]	P	FI	PI-F-C	Degree of Virulence
Beesley[4]	Wild Type	+	+	+	+	Lethal
Otten[5]	Tjiwidej	-	+	+	+	Noninfectious[b]
Girard[1]	EV76	+	-	+	+	Infectious, nonlethal
Burrows[6]	M23	+	+	-	+	Infectious, reduced lethality
Brubaker[7]	G32	+	+	+	-	Infectious, reduced lethality

a. VW = Antigens, P = Pigmentation, FI = Fraction I, PI-F-C = Pesticin-Fibrinolysis-Coagulase.
b. Noninfectious in doses of less than 10^4 organisms.

During our investigation of host-parasite relationships it was necessary to determine the LD_{50} of EV76 by the intravenous route in the mouse. We have found that the lethality of certain attenuated strains is usually greater by this route than by intraperitoneal injection. Doses of tenfold dilutions ranging between 10^3 and 10^7 organisms in 0.1 ml were administered to six mice per point (three males and three females) to determine the appropriate dose levels for an accurate titration. After 3 weeks the resulting curve (Fig. 4) was constructed. As can be seen, the calculation of an LD_{50} was impossible and the dose response was highly unusual. To confirm this finding, a second titration using ten animals per dose and a range of 10^1 to 10^8 cells provided the data shown in Figure 5. High lethality, which was not entirely unexpected, was again noted in the low dose range. Confirmation of the negative dose response is also apparent.

An interesting observation in regard to statistical interpretation of 50% lethality can be made. If the investigator were to test only the high range he would find that EV76 would have an LD_{50} greater than 10^5. If, on the other hand, he challenged animals with low doses, a value of less than 10^2 would be calculated.

It was felt that other P^- organisms should be tested to see if this death response was unique to EV76 or true of any P^- organism.

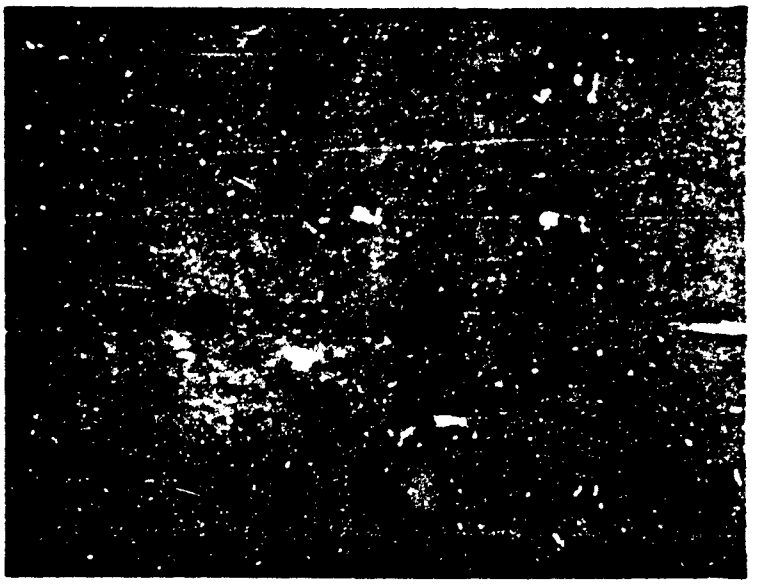


FIGURE 1. Pigmented and Nonpigmented Kim-10
Incubated 5 Days on Hemin Agar at 26 C.

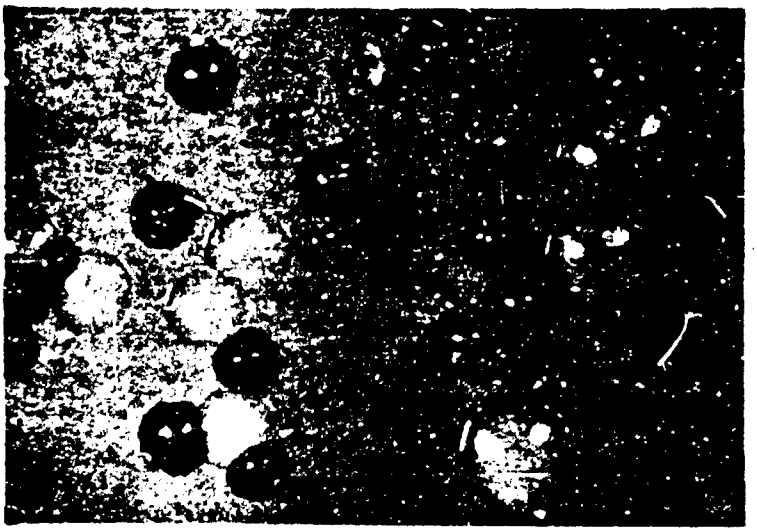


FIGURE 2. Pigmented and Nonpigmented Kim-10
Incubated 4 Days on Congo Red Agar at 26 C.

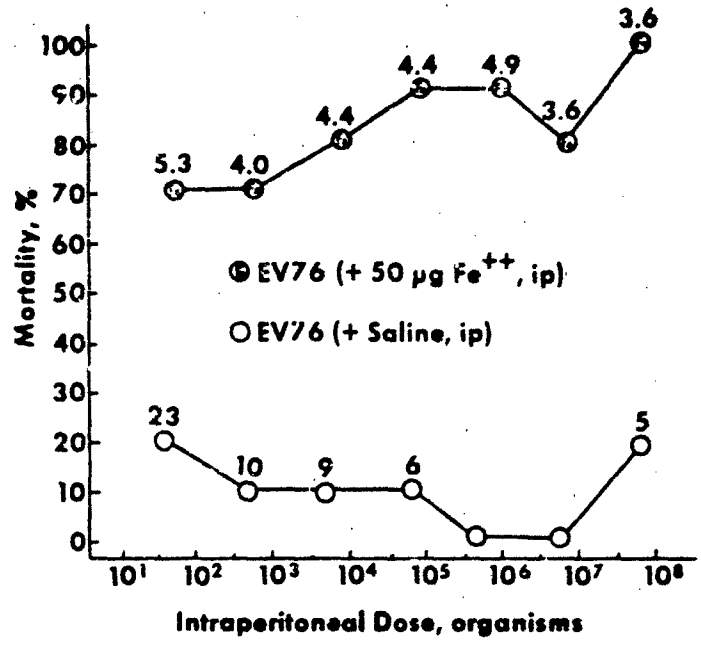


FIGURE 3. Lethality of Live Plague Vaccine (EV76) for Mice by the Intraperitoneal Route with and without Simultaneous Injection of 50 μg of Ferrous Iron. Numerals show average days to death for each dose.

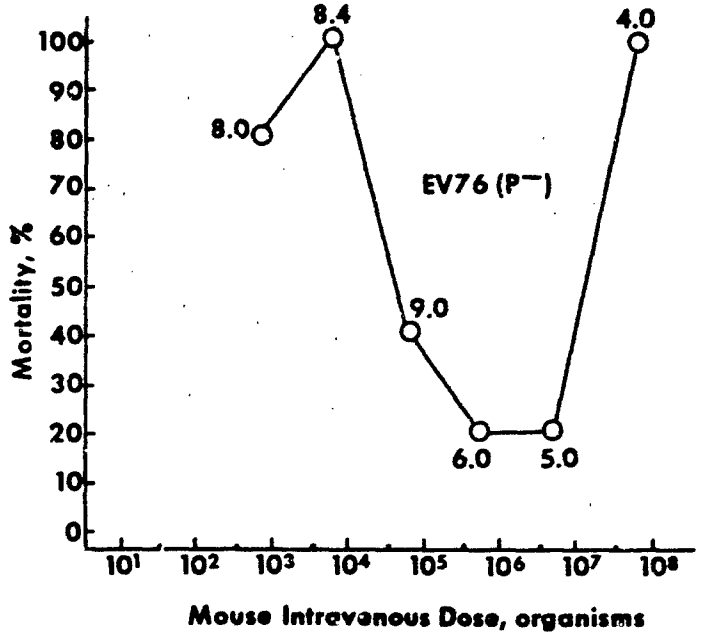


FIGURE 4. Biphasic Response after 30 Days of Intravenously Injected P. pestis Strain EV76 (Experiment 1). Numerals show average days to death for each dose.

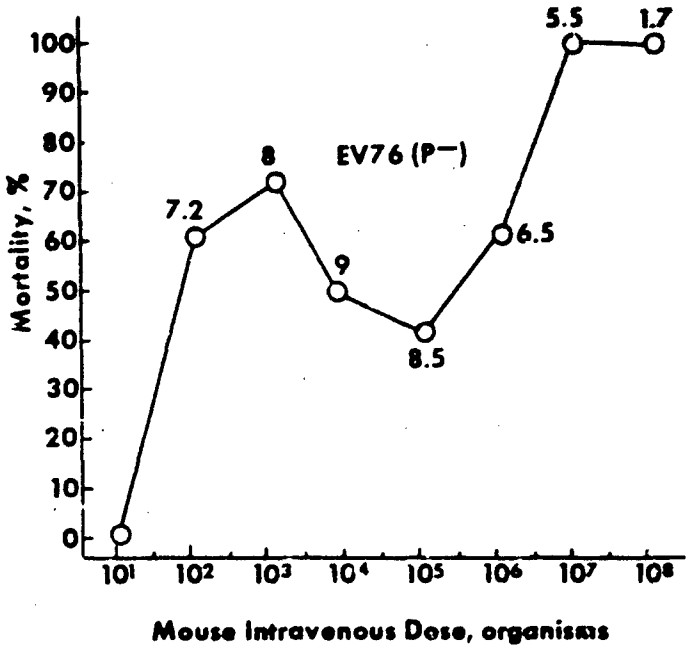


FIGURE 5. Biphasic Response after 30 days of Intravenously Injected P. pestis Strain EV76 (Experiment 2). Numerals show average days to death for each dose.

P^- mutants of P. pestis are easily obtained from fully virulent P^+ parents simply by selecting an isolated white colony from a hemin agar or Congo red agar plate, or by picking a white sector from a P^+ colony (Fig. 6). Nonpigmented strains were isolated from fully virulent Kim-10, partially virulent Fraction I (FI^-) M23, and Pesticin I (Pg^-) G32, then purified by streaking and reisolation. P^- Kim-10 is similar in lethality to EV76 and becomes virulent when injected with iron by the intraperitoneal route. Intravenously it behaves in the same manner as strain EV76, as can be seen in Figure 7. This indicates that attenuation of P. pestis by the loss of the pigmentation factor results in an organism that will kill mice in this fashion and that strain EV76 is not unique.

Strain M23 isolated by Burrows and Bacon[6] is unusual in that it does not produce Fraction I. It is fully virulent for mice but of reduced virulence for guinea pigs. The loss of two or more virulence characteristics usually produces a strain that is essentially avirulent. P^- M23 injected intravenously into mice surprisingly enough produced the data seen in Figure 8. As you can see, this strain was lethal in low doses and it produced a negative death response that was of a lesser magnitude than that of either EV76 or P^- Kim-10. This observation was also noted when an FI^- isolate of Kim-10 PI^- was tested (Fig. 9).

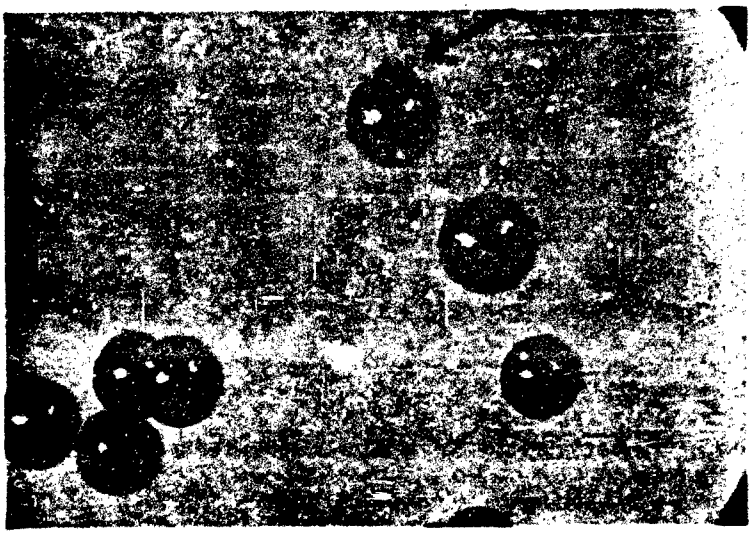


FIGURE 6. Segmented Colony of P. pestis (Kim-10) Showing Mutation to Nonpigmentation.

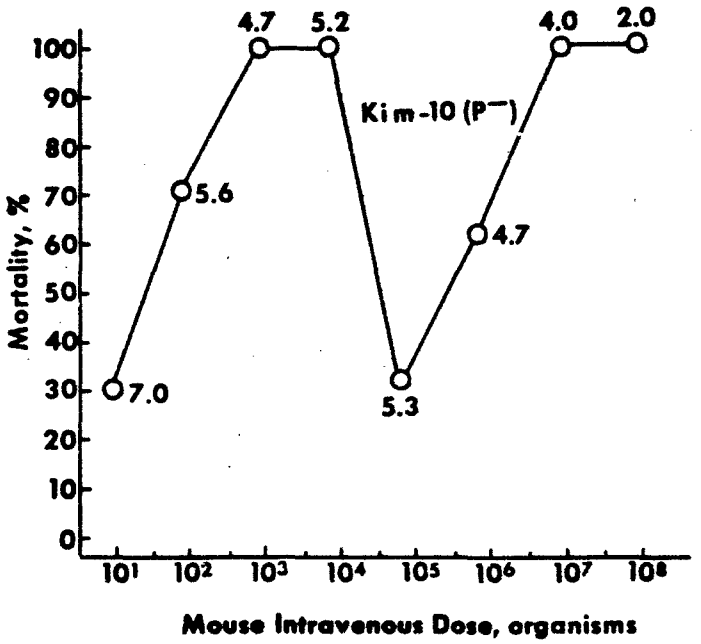


FIGURE 7. Biphasic Death Response in Mice to Intravenous Injection of P. pestis Strain P⁻ Kim-10. Numerals show average days to death for each dose.

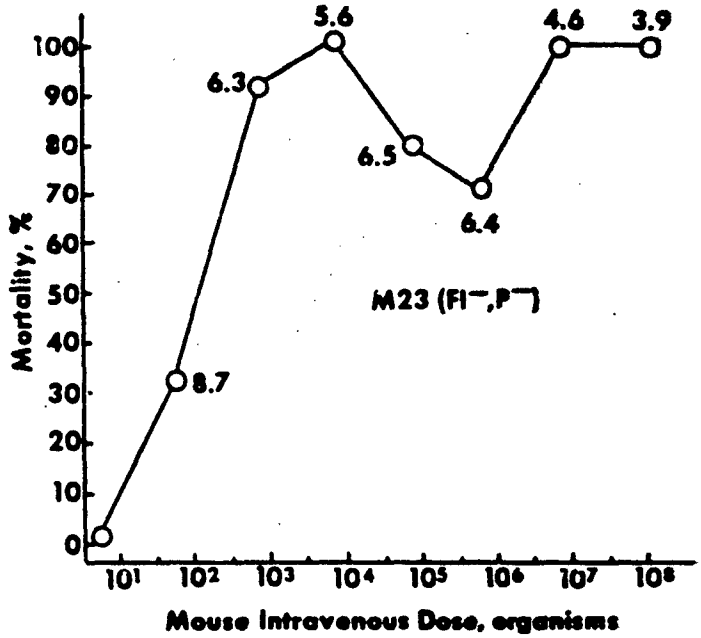


FIGURE 8. Lethality of Fraction I⁻P⁻ P. pestis Strain M23 for Mice Infected by the Intravenous Route.

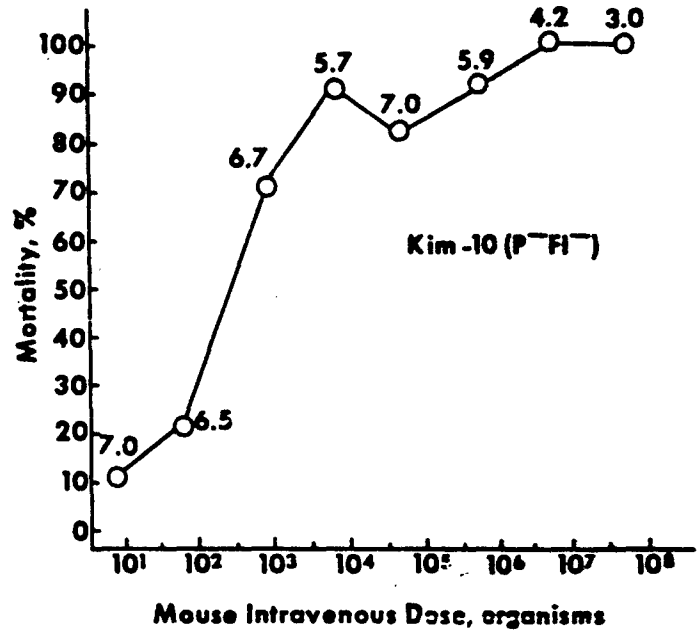


FIGURE 9. Apparent Enhancement of Lethality by Loss of Fraction 1 by P⁻ P. pestis Strain Kim-10. Numerals show average days to death for each dose.

A fourth strain, G32, is unusual because it lacks Pesticin I and genetically linked fibrinolytic factor and coagulase. The loss of this virulence character causes a marked reduction in lethality for the mouse by the intraperitoneal and the subcutaneous routes. By the intravenous route, however, it is fully virulent. Figure 10 shows that, unlike the three strains that have already been discussed, G32 Pg^- is considerably reduced in virulence when the pigmentation factor is lost.

The data presented here are unusual and further studies are needed to elucidate the mechanism or mechanisms involved. It appears that (i) the loss of the virulence factor P^+ is relatively unimportant in the mouse if the infection is initiated by the intravenous route, (ii) the animal is able to survive a moderate challenge but not higher or lower doses, and (iii) the loss of a second virulence factor may or may not result in avirulence.

Explanation of these phenomena awaits further study; however, we might speculate that the high degree of virulence observed in a normally attenuated strain might be due to (i) higher iron levels in the blood-stream, (ii) by-pass of the lymphatic system, or (iii) a combination of the two. The biphasic death response could possibly be due to a stimulation either of specific antibody production by an optimum concentration of bacterial cells or by nonspecific body defenses induced by bacterial endo-toxin in optimum quantity. The data also suggest that Fraction I might be involved, since its absence in P^- M23 and P^- FI^- Kim-10 decreases the negative effect.

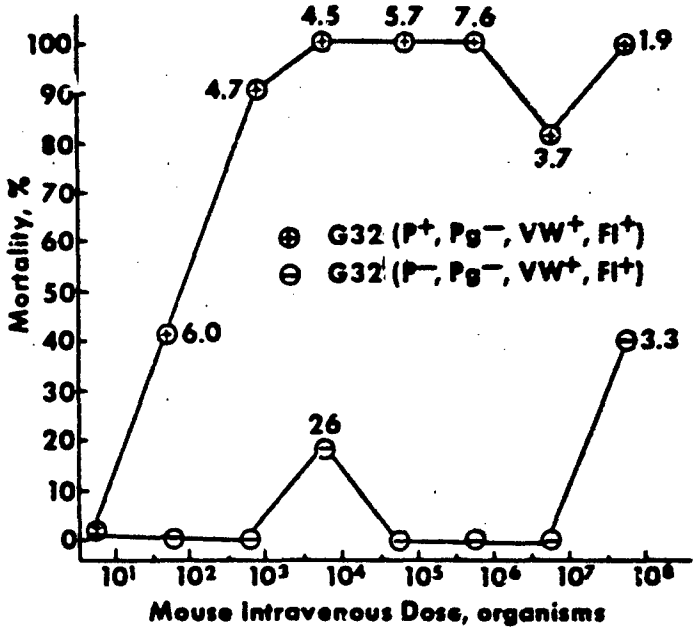


FIGURE 10. Comparison of the Lethality of Pigmented, Pesticin I - Negative P. pestis Strain G32 by the Intravenous Route in Mice with a Nonpigmented Mutant of the Same Strain. Numerals show the average day to death for each dose.

LITERATURE CITED

1. Girard, G. 1963. Immunity in plague infection. Biol. Med. Paris 52:631-731.

2. Jackson, S.; Burrows, T.W. 1956. The pigmentation of Pasteurella pestis on a defined medium containing hemin. Brit. J. Exp. Pathol. 37:560-576.

3. Surgalla, M.J.; Beesley, E.D. 1969. Congo red-agar plating medium for detecting pigmentation in Pasteurella pestis. Appl. Microbiol. 18:834-837.

4. Beesley, E.D.; Brubaker, R.R.; Janssen, W.A.; Surgalla, M.J. 1967. Pesticins: III. Expression of coagulase and mechanisms of fibrinolysis. J. Bacteriol. 94:19-26.

5. Otten, L. 1936. Immunization against plague with live vaccine. Indian J. Med. Res. 24:73-101.

6. Burrows, T.W.; Bacon, G.A. 1958. The effects of loss of different virulence determinants of the virulence and immunogenicity of strains of Pasteurella pestis. Brit. J. Exp. Pathol. 39:278-291.

7. Brubaker, R.R.; Beesley, E.D.; Surgalla, M.J. 1965. Pasteurella pestis: Role of pesticin I and iron in experimental plague. Science 149:422-424.

8. Jackson, S.; Burrows, T.W. 1956. The virulence-enhancing effect of iron on nonpigmented mutants of virulent strains of Pasteurella pestis. Brit. J. Exp. Pathol. 37:577-583.

Unclassified
Security Classification

DOCUMENT CONTROL DATA - R & D
(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
Department of the Army
Fort Detrick
Frederick, Maryland 21701

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP

3. REPORT TITLE
AN UNUSUAL BIPHASIC DEATH RESPONSE IN MICE INFECTED WITH ATTENUATED PASTEURELLA PESTIS

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

5. AUTHOR(S) (First name, middle initial, last name)
Earl D. Beesley
Michael J. Surgalla

6. REPORT DATE
February 1970

7a. TOTAL NO. OF PAGES
17

7b. NO. OF REFS
8

8a. CONTRACT OR GRANT NO.

b. PROJECT NO. 1B662706A072

c. Task-Work Unit 03-005

d. DD 1498 Agency Accession DA OL 0381

9a. ORIGINATOR'S REPORT NUMBER(S)
Technical Manuscript 574

9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
CMs 6629 SMUFD-AE-T 49564

10. DISTRIBUTION STATEMENT
Qualified requesters may obtain copies of this publication from DDC.
Foreign announcement and dissemination of this publication by DDC is not authorized.
Release or announcement to the public is not authorized.

11. SUPPLEMENTARY NOTES
Medical Investigation Division

12. SPONSORING MILITARY ACTIVITY
Department of the Army
Fort Detrick
Frederick, Maryland 21701

13. ABSTRACT

The classical nonpigmented (P^-) plague vaccine strain EV76, which is unable to absorb hematin and exhibits very low virulence for the white laboratory mouse by the intraperitoneal and subcutaneous routes, has been found to be highly virulent when injected intravenously. The lethal dose response curve is biphasic, suggesting that doses in the intermediate range of 10^4 to 10^6 organisms stimulate an effective host resistance mechanism resulting in survival of most of the mice. This peculiar virulence pattern is shown also by nonpigmented variants of strains Kim-10 and M23.

The negative response phase (reflected by survival of infected animals) is less pronounced in mice infected with Fraction I minus (FI^-) organisms such as M23 (P^-FI^-) and Kim-10 (P^-FI^-), suggesting a relationship to FI. Two suggested possible stimuli of the effective host resistance are FI and endotoxin.

14. Key Words

Biphasic death response
Mice
Pasteurella pestis
Attenuated plague vaccine
Vaccine
Negative dose response

DD FORM 1473 REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

Unclassified
Security Classification